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Mammary histology and alveolar cell differentiation during late gestation and early lactation in mammary tissue of beef and dairy heifers

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Abstract

Quantitative histology of mammary parenchymal tissue from 16 Hereford and 16 Holstein heifers was determined for tissue obtained on day 150, 180, and 280 of gestation and on day 49 of lactation. Percent area occupied by stromal tissue was progressively decreased on each consecutive sample day during gestation in Herefords and was lower in both breeds during lactation. Overall, area occupied by stromal tissue elements was also greater in Herefords. Percent lumenal space and number of cells per alveolar cross section was consistently greater for Holsteins and increased across sample periods in both breeds. During lactation more than 40% of the alveolar cells in Herefords were characterized as poorly differentiated, but in Holstein heifers nearly all of the cells were classified as either intermediate or fully differentiated. These data provide additional evidence to support the idea that much of the difference in milk production between beef and dairy animals depends on increased mammary function. Whether differences in milk production within dairy breeds are also explained by altered cellular differentiation remains to be determined.
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Keywords: Mammary gland; Breed; Udder; Differentiation; Gestation; Mammary histology

1. Introduction

Increases in milk production for dairy cows during the second half of this century have been dramatic. There are many reasons for this change. Among these are: improvements in genetic selection methods and associated use of AI, attention to nutritional needs and ration formulation as well as concern with mastitis control and milking management. Regardless, milk production at the animal level ultimately depends on the function of the mammary gland. The focus of this paper is the udder and specifically these questions.

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Has the structure or function of the bovine mammary gland been impacted by long-term selection for increased milk production? We address this question by comparing breeds with very different milk production potentials as well as divergent selection criteria i.e. milk vs. meat.

We utilized mammary tissue samples collected from Hereford and Holstein heifers from a study described by Keys et al. (1989). Our first objective was to quantify the histological development of parenchymal tissue during gestation and into lactation with a primary focus on comparative development between these breeds. A second objective was to determine if previously reported differences in milk production between breeds were related to cytological differentiation of the secretory epithelium among lactating animals. Our hope was that morphometric characterization of mammary tissue might indicate the impact of milk production potential on development of mammary parenchymal tissue and specifically the impact of milk production on secretory cell differentiation.

2. Materials and methods

2.1. Experimental design

As reported by Keys et al. (1989), 16 Hereford and 16 Holstein heifers were housed and managed under identical conditions. Animals were also treated in accordance with approved animal care procedures. Equal numbers of heifers were slaughtered at 150, 180 and 260 days of gestation and at 49 days of first lactation. Lactating animals were machine-milked twice daily. At the time of slaughter (3 h or less from the time of the last milking) udders were removed to determine parenchymal and stromal tissue mass and to measure udder DNA, RNA, fat, and protein (Keys et al., 1989). Three to six samples of parenchymal tissue (~2 mm cubes) were also taken from a randomly selected mammary gland of each animal, placed in fixative, and subsequently processed for histological evaluation as described in prior publications (Akers et al., 1981b; Nickerson and Akers, 1983). Specifically, samples were taken from each of three zones located along an imaginary path oriented in a vertical axis from the center of the teat to the margin of the parenchymal tissue adjacent to the ventral body surface. The first of the zones was located just above the gland cistern, the second about mid-way and the third near parenchymal border.

Two slides were prepared from each tissue sample and stained with Azure II (Akers et al., 1981b). The slides were coded to prevent observer bias and the sections evaluated microscopically to determine the proportions of tissue area occupied by epithelium, stroma, or lumen. Morphometric procedures were essentially as described in prior publications (Akers et al., 1981b; Smith et al., 1989; Capuco et al., 1997). Briefly, sections were examined using a 40× objective lens using a microscope equipped with a grid positioned in the eyepiece ocular. Each of the 36-grid interactions per view were classified (stroma, lumen, or epithelium) depending on the structure or tissue element located under the grid intersection. For tissue collected from each zone, six random fields were evaluated from two independent tissue sections. Thus for each zone 216 intersections were classified or 648 per animal.

We also determined the relative cytological differentiation of the alveolar secretory cells for tissues collected from lactating animals. For this analysis, in addition to determining the tissue type at a grid intersection, when an epithelial cell was encountered the cell was further classified relative to its differentiation status. Cells were characterized as (E1) poorly differentiated, (E2) intermediately differentiated or (E3) fully differentiated as described previously (Akers et al., 1981b; Nickerson and Akers, 1983). Examples of secretory cells with these characteristics are illustrated in Fig. 3. Briefly, secretory cells classified as fully differentiated are characterized by the presence of many vacuoles, rounded basally displaced nuclei, abundant cytoplasm, and frequent large apical lipid droplets. Intermediately differentiated cells had fewer vacuoles, more irregularly shaped nuclei, and greater nuclear to cytoplasmic ratios. Poorly differentiated cells were characterized as having few if any vacuoles, large or randomly positioned lipid droplets, and little relative cytoplasmic area.

In addition, we also determined the number of cells per alveolar cross section. For this measurement, we used only the tissue samples collected from the middle parenchymal zone since the initial analysis indicated no statistically significant difference between zones. We selected alveoli, which appeared to be sectioned

approximately through the center of the alveolus. This was based on the relative uniformity of the profile and appearance of the individual secretory cells of the alveolus and the lack of epithelial cells in the lumenal space which might indicate that the section was cut near the periphery of the alveolus. For each animal 10 to 20 alveoli were selected.

2.2. Statistical analysis

ANOVA was performed with the PC-SAS software package (Ver. 8.2, SAS Inc., Cary, NC), using PROC GLM. Data were analyzed using a model that included main effects for Breed, Day, and Zone as well as two and three way interactions. Breed and Day and Breed × Day interaction were tested with the Cow (Breed × Day) term. The effects of zone and interactions were tested with the overall error term. Data to evaluate differentiation of the secretory cells during lactation were similarly evaluated but with Breed as the only main effect.

3. Results

3.1. Quantitative histology

Because the effect of zone was not significant for percent tissue area occupied by epithelium, stroma or lumen, data were averaged for zones. For the epithelial area measurement there was a significant effect of day ($P<0.01$) but no overall effect of breed ($P<0.7$). Tissue area occupied by epithelium was significantly lower ($P<0.05$) in both breeds during lactation (Fig. 1). For the lumenal area there were significant effects for breed ($P<0.001$) and day ($P<0.001$) but their interaction was not significant. Average lumenal area was consistently greater for Holstein heifers and increased in both breeds during lactation (Fig. 1). Effects of breed ($P<0.001$) and day ($P<0.001$) were significant for the area occupied by stromal tissue but there was no significant interaction. There was relatively little change in stromal tissue area in Holstein heifers until lactation but a more progressive decline from gestation into lactation for Hereford heifers (Fig. 1) and the relative difference between breeds was greatest during lactation. The number of alveolar cells per cross-section was

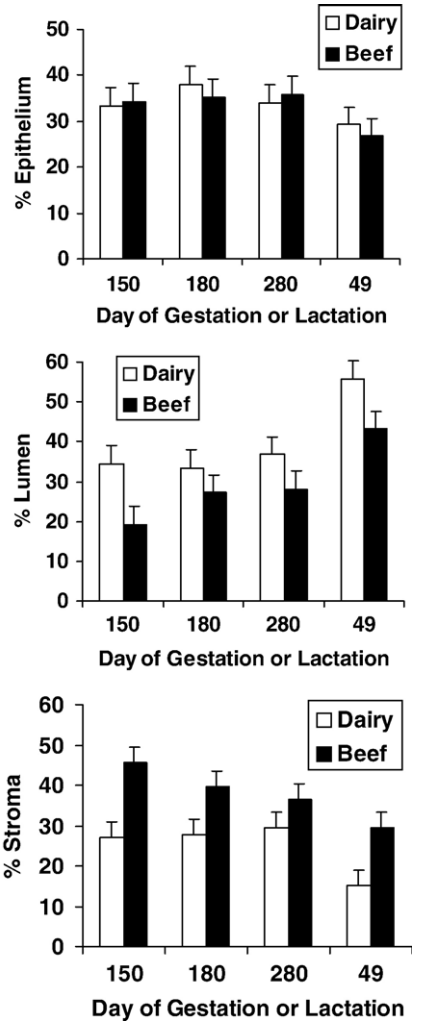

Fig. 1. Quantitative histology of mammary parenchymal tissue for Hereford (beef) and Holstein (dairy) heifers during gestation and on day 49 of lactation. Tissue samples from each of three zones in the udder were used to determine the percent area occupied by epithelial cells (upper panel), stromal tissue (middle panel), or lumen space (lower panel). Data are averaged for zones and are least square means ± S.E.

consistently greater ($P<0.01$) in Holstein heifers but both breeds showed an increase over time ($P<0.01$) and the difference between breeds was most striking during lactation (Fig. 2).

3.2. Cytological analysis of alveolar cells in lactating heifers

While about half of the cells epithelial cells in both breeds were classified as intermediately differentiated,

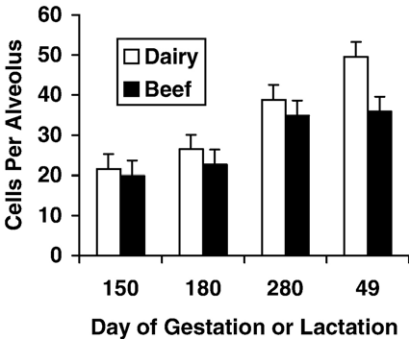

Fig. 2. Mean number of epithelial cells per alveolar cross section in mammary tissue for Hereford (beef) and Holstein (dairy) heifers during gestation and on day 49 of lactation. Data are given as least square mean ± S.E.

the proportions of fully and poorly differentiated cells were essentially reversed between breeds (Table 1 and Fig. 3). Slightly more that half of the epithelial cells in Holstein heifers were classified fully differentiated. Consequently, nearly all the epithelial cells of the Holsteins were either classified as fully or intermediately differentiated. For the Herefords, more than 40% of the cells had little evidence of secretory activity. These poorly differentiated cells were corresponding rare in Holstein heifers.

4. Discussion

Holstein and Hereford heifers were managed identically and machine milked twice daily. During the month prior to calving, both groups of heifers were periodically moved through the milking parlor to allow the animals to acclimate to the lots, handling, and noises of the milking parlor. Since neither Holstein nor Hereford heifers had been previously milked, training to the milking machine after calving was not different between the breeds. Furthermore,

Table 1
Percentage of alveolar epithelial cells in each of three differentiation classes in lactating Holstein and Hereford heifers

Differentiation class	Breed		
	Beef	Dairy	S.E.M.
Poor (E1)	44.5	1.2[a]	4.2
Intermediate (E2)	54.8	47.3	4.0
Full (E3)	1.3	52.9[a]	1.7

[a] Means with superscript differ at $P<0.001$.

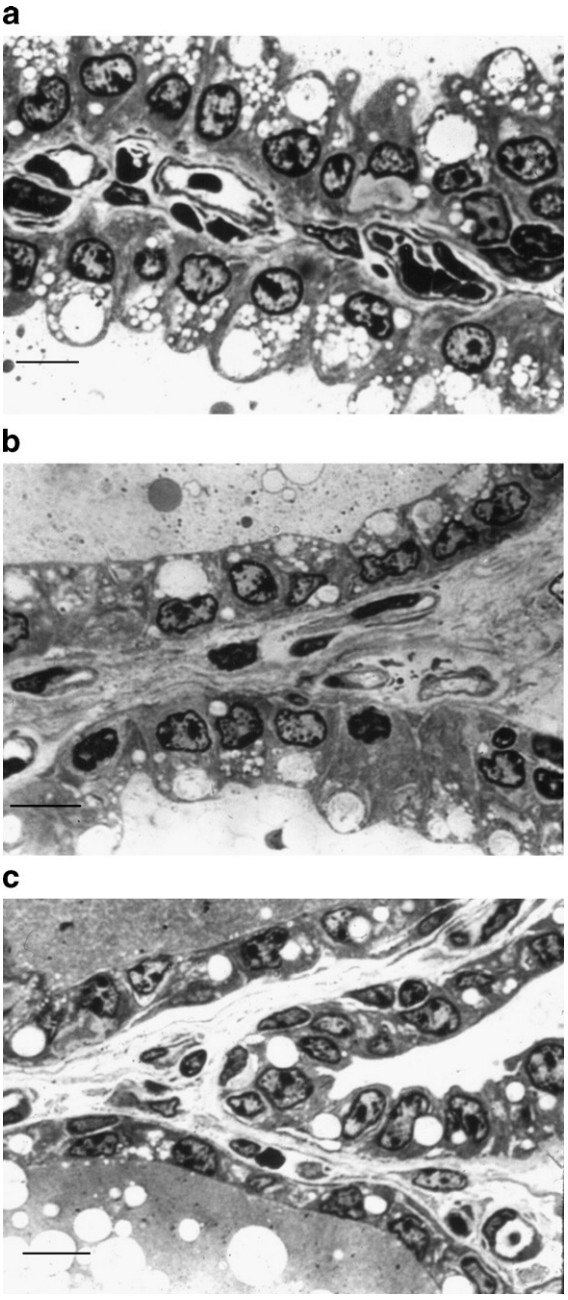

Fig. 3. Characteristics of full (a), intermediate (b), and poorly differentiated mammary (c) epithelial cells are illustrated. Briefly, fully differentiated secretory cells are polarized, exhibit abundant secretory vesicles, basally located nuclei, and a large cytoplasmic to nuclear ratio. Poorly differentiated secretory cells are less polarized, exhibit minimal numbers secretory vesicles and frequently have large lipid droplets as well as a small cytoplasmic to nuclear ratio. The bar in each panel is equivalent to 10 μM.

there were no apparent differences in milk letdown or milking behavior between the breeds. There were no attempts to measure residual milk in these animals. These observations are important. Boutinaud et al. (2003) showed that milking frequency can impact the number of cells per alveolus in lactating goats and have subsequently reviewed mammary tissue factors that impact milk production potential (Boutinaud et al., 2004). In addition, Singh et al. (2005) reported that milk accumulation could induce secretory cell death. These are important considerations but we have no evidence to suggest that these factors influenced the results of this study.

As discussed in a review (Akers, 2000), differences in average milk production (3.5 vs. 20.3 kg/day) for Hereford vs. Holstein are likely explained by both differences in cell number (DNA content) and function per cell (RNA/DNA) (Keys et al., 1989). The data presented in this report focus on characteristics of the mammary tissue rather than determinations of total udder content of cells or parenchymal tissue metabolism as reported by Keys et al. (1989). However the data provide an important adjunct to support these overall conclusions. Specifically, these data suggest that our cytological classification of epithelial cells is a meaningful and relevant measure of mammary tissue function during lactation. It seems likely that selection for increased milk production among Holsteins promotes both increased cellular differentiation as well as increased cell proliferation but confirmation will require study within rather than between breeds.

Because changes in tissue development during gestation are not specifically tied to functionality at each sample period, it is more difficult to interpret the physiological significance of relative change in histology between breeds or between sample days during gestation. Nonetheless, the consistent greater lumenal space (Fig. 1) and number of cells per alveolar cross-section (Fig. 2) for Holsteins vs. Herefords suggests that there may be inherently greater proliferation potential of secretory cells in Holsteins. However, it is important to consider mammary histology in the context of development. It should be appreciated that defined lobules and alveoli were apparent in both breeds at the time of the first sampling on day 150 of gestation. Thus, there is little reason to expect dramatic changes in mammary tissue structure. Consequently, further development up to the time of parturition largely represents a ‘maturation’ of structures already part of the parenchymal tissue structure. This, however, does not preclude breed or between animal differences, in the final size of alveoli, capacity of the secretory cell to fully differentiate or accumulation of secretions with onset of lactogenesis.

For both breeds across the sampling days of gestation, percent area occupied by stroma, epithelium, and lumen averaged 33%, 34%, and 32%, respectively. This is similar to values previously reported for dairy cattle (Akers et al., 1981b; Capuco et al., 1997) and sheep (Smith et al., 1989). If tissue from non-pregnant postpubertal Holstein heifers is considered, corresponding averages are 84%, 12%, and 3% (Sejrsen et al., 1982). Clearly, this difference is largely a reflection of the lack of lobulo-alveolar development the younger heifers. Regardless, some indication of tissue development comes from considering the relative changes in area occupied by epithelium, stroma, and lumen during gestation in the current experiment. For example, between days 150 and 280 of gestation area occupied by stromal tissue in Hereford heifers decreases from 46% to 39%. This likely represents not a true disappearance of stromal cells but rather increasing lumenal area as the number and volume of alveoli increase. Between day 280 of gestation and day 49 of lactation, there is a further decease of stromal area. This likely indicates a further increase in alveolar area and accumulation of secretions with the onset of lactation. In support of Capuco et al. (1997) there was virtually no change in stromal area in Holstein heifers until lactation. Relative changes in lumen space support this idea. Namely, there is little change in lumenal area for dairy heifers until lactation but nearly a doubling for beef heifers. This suggests that mammary tissue is relatively more developed (i.e. more extensive lobulo-alveolar structure) earlier in gestation in Holstein compared with Hereford heifers. Since a greater proportion of stromal tissue is evident in at the earliest sample period for Herefords, this may also reflect developmental events that occurred prior to gestation.

Paradoxically, area occupied by epithelium changes little in either breed during late gestation and is actually lower during lactation. This is most likely an artifact of the dramatic increase in lumenal space and corresponding compression of epithelium and stromal tissue with the onset of milk secretion. Since there are only small changes in tissue concentrations of DNA in

the periparturient period (Akers et al., 1981a; Capuco et al., 1997), it is unreasonable to suggest that actual numbers of epithelial cells on a tissue basis decrease. This does not diminish the possibility for differences in rates of cell turnover but it seems this would be more of a factor in subsequent lactation cycles. Averaged across breeds, cells per alveolar cross-section double between day 150 of gestation and day 49 of lactation (20 vs. 43). Moreover, there was a small but consistent increase in number of cells per alveolar cross section in dairy heifers. This difference between breeds is greatest during lactation (36 vs. 49 cells per alveolus; $P<0.05$). In lactating goats the average number of cells per alveolar cross-section for goats milked 3 times a day was 28.4 (Boutinaud et al., 2003). Smith et al. (1989) reported an average of 36.6 cells per alveolar cross section for samples collected from ewes just before lambing. Thus there may be inherent differences between species. Nevertheless, the difference in alveolar size between beef and dairy heifers likely explains a part of the greater total udder mass and therefore functional capacity of mammary glands of Holsteins compared with Herefords (Keys et al., 1989).

While these differences seem relatively small, it is instructive to consider the impact of alveolar diameter on alveolar volume. If the alveolus is assumed to be a sphere, alveolar volume can be calculated from the following formula: $V=4/3\pi r^3$. If the radius of an alveolus is 50 μm, the volume will be 523,599 μm^3. An increase of only 10 μm nearly doubles the volume (904,779 μm^3). This is the approximate width of an epithelial cell. Moreover, the area occupied by the lumenal space is also particularly increased for lactating Holsteins compared with Herefords. These observations suggest that a difference in alveolar volume and consequently, storage capacity, could be important in the selection for increased milk production. Of course, this is based on the assumption that comparisons between beef and dairy heifers mirror selection responses of dairy heifers.

5. Conclusions

The relative failure of cytological differentiation in mammary tissue of beef compared with dairy heifers is puzzling since milking stimulation and management was identical between breeds in this study. Is it possible that selection for increased milk production has allowed for the maximization of differentiation signals during the critical periparturient period? Whether similar differential effects are apparent when Holsteins selected for milk production are compared Holsteins not selected (or less intensely selected) remains to be determined. These data provide additional evidence to support the idea that much of the difference in milk production between beef and dairy animals depends not only on increased parenchymal mass in dairy animals but also in the enhanced activity of the majority of individual secretory cells.

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